STRUCTURAL CHANGES IN BISCUITS MADE WITH CELLULOSE EMULSIONS AS FAT REPLACERS

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SHORT TITTLE=Cellulose emulsions in low fat biscuits

Abstract

Biscuits are a popular baked cereal food much appreciated by consumers. In the last few years, cellulose derivatives have been successfully used as fat replacers in biscuits. In this way, not only is the total amount of fat reduced, but also the saturated fatty acids and the trans fatty acids are eliminated. The aim of this study is to increase understanding of the functionality of different cellulose ether emulsions used as fat replacers in biscuits. For this purpose, three emulsions with different cellulose ethers were designed: hydroxypropyl methylcellulose (HPMC), methylcellulose (MC) and methylcellulose with greater methoxyl substitution (MCH). The microstructure and textural properties of the doughs and biscuits prepared with these emulsions were studied and the effects of cellulose types and glycerol as textural improver were also analyzed. The results showed that the incorporation of glycerol in the doughs made with MC and HPMC cellulose emulsions seems to make the dough softer, bringing the values closer to those of the control dough; however, this effect disappears once the dough is baked. The presence of glycerol does not seem to have an effect on the hardness of the doughs and biscuits made using the MCH emulsion.

Key words: biscuits, fat replacement, celluloses, structure.

INTRODUCTION

Biscuits are a popular baked food very appreciated by consumers because of their pleasant taste and texture. However, one important problem of such baked goods is their fat and sugar content, which turns them into high-calorie products, at a time when consumers are becoming increasingly interested in healthy food products.

Fats improve the texture, appearance, lubricity, mouthfeel and taste, thus contributing to food palatability (Drewnowski 1992; Grigelmo-Miguel et al., 2001; Zoulias et al., 2002a). Also, they provide bulk to foods, retain water, facilitate heat transfer mechanism at elevated temperatures (Drewnowski et al., 1998) and increase the feeling of fullness during the meal (Leland, 1997). In biscuits, higher percentages of fat produce tenderer biscuits with less hard texture and more inclined to melt in the mouth (Lai and Lin, 2006).

As regards fat, the focus is on low-fat foods, low fat saturated food and the absence of trans fatty acids; therefore, due to the important functionality of fat in biscuits, achieving a reduction in the fat content without affecting quality properties or consumer acceptability is a challenging task. Fat replacement in biscuits by inulin, maltodextrin, polidextrose and different commercial fat mimetics, such as Oatrim™, Simplesse™ or Litesse™ has been studied by many different authors with various levels of success (Inglett et al., 1994; Oreopoulou and Tzia, 2002; Zoulias et al., 2002a, 2002b;

Zbikowska and Rutkowska, 2008; Röβle et al., 2011; Laguna et al., 2012; Rodríguez-García et al., 2013).

In the last few years, cellulose derivatives, such as HPMC, have been successfully used as fat replacers in biscuits (Laguna et al., 2014); the fat content has been successfully reduced in biscuits by means of a cellulose ether emulsion as a shortening replacer. This cellulose ether emulsion is made of a liquid vegetable oil and has a lower fat content and saturated fatty acid content than a conventional margarine or butter. The consistency provided by the cellulose emulsion makes it possible to incorporate liquid oil into the biscuit recipe and provides a good consistency for manipulating the biscuits (laminate, cut and baking) in the same way as a full fat recipe would. In this way, not only is the total amount of fat reduced but also the saturated fatty acids and the trans fatty acids are eliminated. Biscuits prepared with the cellulose emulsions have good consumer acceptability (Tarancón et al., 2013b, 2014a, 2014b).

For the purposes of better understanding the functionality of using cellulose ether emulsions as fat replacers, various pieces of research have been carried out. The effect of using a variety of cellulose ether emulsions, instead of a conventional shortening, on the changes occurring in a dough biscuit recipe during heating were studied by Sanz et al. (2015a). The linear viscoelastic and textural properties after different heating times were studied and compared: in the shortening dough, the changes during heating were mainly governed by the fat melting process, while in the emulsion dough these changes were associated with the cellulose thermo-gelling properties. However, the structural features, which explain this functionality, are still not well understood.

On the other hand, it is known that cellulose ether substitution exerts a definitive role on the emulsion properties and their thermal behavior. The thermal rheological properties, particle size distribution and the microstructure of emulsions prepared with different types of cellulose ethers which varied in terms of the degree of methoxyl/hydroxypropyl substitution were studied. After heating, the methylcellulose emulsion with the highest methoxyl content demonstrated syneresis, fat flocculation and the appearance of particle size polydispersity, indicating a lower thermal stability and less thermal reversibility (Sanz et al., 2015b). However, it would be interesting to ascertain how these types of cellulose behave when they are heated during the processing of a food.

The aim of this study is to increase understanding of the functionality of different cellulose ether emulsions used as fat replacers in biscuits. The microstructural and textural properties of the doughs and biscuits prepared with these types of emulsions were studied and related. The effect of cellulose ether chemical substitution and the effect of glycerol used as a textural improver were also analyzed.

MATERIALS AND METHODS

Emulsion preparation

Three different cellulose ethers with different thermogelling ability (F4M, a hydroxypropyl methylcellulose (HPMC), (A4M (MC) and MX (MCH), methylcelluloses where MX is a methylcellulose with greater methoxyl substitution than A4M and higher molecular weight (The Dow Chemical Co.) were used to prepare oil-water-cellulose emulsions. Sunflower oil with high levels of oleic acid (Carrefour, Madrid, Spain) (47%), water (51%) and the different cellulose ethers (2%) were the ingredients used to prepare the emulsions following the procedure used by Sanz et al. (2015b).

Biscuit preparation

The ingredients used in dough preparation were: soft wheat flour suitable for biscuits 100% (Belenguer, S.A., Valencia, Spain) (composition data provided by the supplier: 11% protein, 0.6% ash; alveograph parameters P/L=0.27, where P is the maximum pressure required (calculated as resistance to stretching in mm) and L is the extensibility (mm); and W=134 J, where W is the baking strength of the dough), shortening 35% (St. Auvent, Vandemoortele France, 78.4% total fat, 51% saturated fatty acids,: 20% monounsaturated fatty acids, 6% polyunsaturated fatty acids and <2% trans fatty acids) or cellulose emulsion as shortening replacer 35%, sugar 25% (Azucarera Ebro, Madrid, Spain), milk powder 1.8% (Central Lechera Asturiana, Peñasanta, Spain), salt 1%, sodium bicarbonate 0.4% (A. Martínez, Cheste, Spain), ammonium hydrogen carbonate 0.2% (Panreac Quimica, Barcelona, Spain) and tap water 9%. In the formulations with a shortening replacer, the doughs were prepared with and without glycerol (3.2 %) (Panreac Quimica, Barcelona, Spain).

Biscuit of 50 mm in diameter and thickness of 3.4 mm were prepared as explained by Tarancón et al. (2013a). The dough and biscuit samples were evaluated on the following day in every case. Seven different biscuits were prepared: control without fat replacement, MC elaborated with methylcellulose, HPMC elaborated with hydroxypropyl methylcellulose and MCH elaborated with the methylcellulose with the highest degree of methoxilation; the last three samples were prepared with and without glycerol as texture improver.

Texture

A TA-XT.plus texture analyzer equipped with the Texture Exponent software (version 2.0.7.0. Stable Microsystems, Godalming, UK) was used to evaluate dough and biscuit texture. The test speed was always 1 mm s^{-1} and the trigger force was 0.098N. The test was conducted on six replicates of each formulation.

The penetration tests were conducted with the upper Volodkevich Bite Jaw (VB), penetrating the dough disc (34 mm in thickness with a diameter of 50 mm) or biscuit (50 mm in diameter) to 2.5 mm. The maximum force (N) of penetration was measured.

Microstructural analysis

Confocal Laser Scanning Microscopy (CLSM)

Different doughs and emulsions were observed using a Nikon confocal microscope C1 unit that fitted on a Nikon Eclipse E800 microscope (Nikon, Tokyo, Japan). Rhodamine B and Nile Red were used as fluorescent dyes. For sample visualization, the microscope slide and the samples were prepared as Rodriguez-García et al. (2013) and the images were stored using the microscope software (EZ-C1 v.3.40, Nikon, Tokyo, Japan).

Cryo Scanning Electron Microscopy (Cryo-SEM)

For Cryo-SEM observation, a Cryostage CT-1500C (Oxford Instruments Ltd., Witney, UK) was used, coupled to a JSM-5410 scanning electron microscope (Jeol, Tokyo, Japan) following the protocol used by Rodriguez-García et al. (2013).

Sensory Analysis

The sensory analysis was carried out in a standardized test room (ISO, 2007). A total of 83 untrained panelists (consumers) aged between 15 and 64 years old, who frequently consumed this type of biscuit, took part in the study. Each consumer received four biscuits (the control and one for each shortening replacer with glycerol) presented individually in a single session following a balanced complete block experimental design. The biscuits were coded with random three-digit numbers. Consumer acceptance testing was carried out using a categoric nine point hedonic scale (9: like extremely and 1: dislike extremely). The consumers had to score first their liking for the 'odour' and 'colour', and after eating the sample their liking for 'hardness', 'crispness', 'taste', 'sweetness' and 'overall acceptability' for each biscuit sample. Consumers were asked to rinse their mouths with water between each sample. Data acquisition was performed using Compusense® five release 5.0 (Compusense Inc., Guelph, Ontario, Canada).

Statistical analysis

An analysis of variance (two-way ANOVA) with fixed factors (sample, glycerol and the interaction sample x glycerol) was applied to study the effects of the type of cellulose and the presence of glycerol on the different instrumental and sensorial parameters. The least significant differences were calculated by the Tukey test and the significance at $p < 0.05$ was determined. These analyses were performed using XLSTAT 2009.4.03 statistical software (Addinsoft, Barcelona, Spain).

RESULTS AND DISCUSSION

Biscuit dough

Texture

Figure 1 shows the textural curve profile obtained after the penetration test performed on the biscuit doughs made without (A) and with glycerol (B); when there is no glycerol in the formulation, the maximum force values of the doughs made using cellulose emulsions as fat replacers are higher than those of the control dough. However, the maximum force value falls and it is closer to that of the control when there is glycerol in the dough formulation, which leads to softer doughs that are easier to handle. Table 1 shows the maximum force values for each dough. In the light of the results, it may be said that the formulations made using cellulose with or without glycerol had significantly higher force values ($p<0.05$) than the control dough. In the case of those doughs made without glycerol, MCH was the dough whose values were the most similar to the control, whereas in the case of those dough's made with glycerol, it was HPMC which were the most similar to the control, as can be seen in the textural profiles (Figure 1). Incorporating glycerol into the dough formulations seems to attenuate the rise in the maximum force produced by replacing the original fat by the MC and HPMC emulsions. Nevertheless, the glycerol does not have a significant influence ($p>0.05$) on the hardness of the doughs formulated using MCH.

Confocal Laser Scanning Microscopy (CLSM)

Figure 2 shows the micrographs of the HPMC, MC and MCH emulsions. The fat globules are dyed green and the continuous phase (cellulose ether and water) red.

The microstructure shows a dense matrix of stable fat globules, corresponding to the dispersed phase, immersed in a continuous phase made up of water-hydrated cellulose. The long chains of polymers, methylcellulose or hydroxypropylmethylcellulose, form a three-dimensional network that compartmentalizes the continuous aqueous phase and immobilizes the oil globules or dispersed phase. As the movement of fat particles is prevented, it is unlikely that two drops approach each other and aggregate or fuse (Aranberri et al. 2006), avoiding decomposition mechanisms in the emulsions, such as flocculation or coalescence (Piorkowski and McClements, 2014). In Figure 2, it can be seen that the emulsions have fat globules of differing sizes, with the MCH emulsion being the one that has the biggest globules. In all likelihood, the high molecular weight of the MC cellulose, permits the formation of networks or large compartments, which may favor the formation of large globules. As for the shape of the globules, the fat globules in the HPMC emulsion are much more defined than those in the MC and MCH emulsions whose globules are much more irregular in appearance.

In addition, by using this technique, it is possible to find out the distribution of the ingredients in the structure of the biscuit dough. When the sample is treated using the contrast dyes Rhodamine and Nile Red, the proteins and carbohydrates go red, the fat goes green and the starch goes black (Figure 3). In the micrograph of the control dough (Figure 3A), a continuous matrix, dyed red-orange, can be seen. One part of the fat phase seems to be fused with the continuous phase which is mainly made up of gluten, milk protein and carbohydrates; the starch granules, colored black, are dispersed in this matrix. The fat, colored green, is found in a reticular formation around the starch granules, chiefly forming independent blocks. This structure is similar to that previously described by Chevallier et al. (2000).

The emulsion in the dough made using HPMC (Figures 3B and C) appears in the form of small green or black globules that are dispersed in the matrix, and it is distributed more homogeneously than in the doughs made using the methylcelluloses, MC and MCH (Figures 3D, E, F and G). In the latter, the emulsion can be observed in the form of dark blurred zones, distributed irregularly in the dough, which encompass small fat globules.

In the case of the doughs formulated using HPMC and glycerol (Figure 3C) one part of the emulsion is more tightly fused with the continuous phase than in the doughs that do not contain glycerol. Thus, in the dough with glycerol an intense orangey tone can be observed in the continuous phase which is due to the effect of the fat fusing with the continuous phase. If the two doughs made using the methylcelluloses with or without glycerol (MC and MCH) are compared, in those formulated with the MCH emulsion, the fat is freer (more free fat globules, dyed green, can be appreciated outside the dark areas corresponding to the emulsion). The fusion of the free fat in the continuous phase (as in the case of HPMC +glycerol) and the free fat globules observed in the MCH emulsion (with or without glycerol) prepared doughs may be related to the lower force values obtained in the textural analysis, which were more similar to that obtained for the control doughs. This may be due to the texturizing properties attributed to the free fat, which acts as a lubricant surrounding the starch granules and preventing the gluten from developing a cohesive, extensible and strong network.

Cryo Scanning Electron Microscopy (Cryo-SEM)

Figure 4 shows the microstructural images of the interior of the biscuit dough obtained by Cryo-SEM. In the control dough (Figure 4A), the starch granules are embedded in a protein-sugar system. These results coincide with the microscopic observations of other authors, as explained by Baltsavias et al. (1999) in their study in which the structure of the short-dough biscuits consists of a mixture of proteins and starches and where the fat acts as a filler at low concentrations.

The presence of glycerol leads to the dough having a much more compact and uniform matrix; this is mainly appreciable in the doughs prepared using HPMC and MC (Figures 4B, C, D and E), whereas the dough made with MCH (Figures 4F and G) is the one where this difference is least appreciable. This coincides with the textural results in which no difference may be appreciated between the hardness of the dough's made using MCH with and without glycerol. In the case of the dough's made using both the HPMC emulsion and MC with glycerol (Figures 4C and E), the fat combines with the starch granules to a much greater degree, lubricating the dough more than in the other samples, which could affect its texture and lead to softer dough's, as has already been explained.

Biscuit

Texture

Figure 1 shows the textural profiles obtained after the penetration tests, corresponding to the force needed in the first bite of the biscuit, made without (C) and with glycerol (D). As may be seen, the textural profiles of the biscuits made using the MC and HPMC emulsions had higher values of hardness than the control biscuit, regardless of whether glycerol was present or not. However, the textural profiles of those biscuits made with the MCH cellulose were very similar to the control biscuit in terms of their hardness and crispness, as may be appreciated by the presence of a greater number of force peaks registered along these two curves throughout the penetration test.

Maximum rupture force parameters (Table 1) were calculated using the textural profiles for the purposes of achieving a better analysis of these textural differences.

The results indicated that more force was needed to break the biscuit in the case of those made using the MC emulsion, whether glycerol was present or not. Of all the celluloses, MCH was the one whose values of hardness were seen to be similar to those of the control biscuit ($p>0.05$). It may also be seen how in none of the samples the presence of glycerol produced any significant differences in terms of hardness. What seems to be a determining factor as regards the hardness of biscuits is the type of emulsion used, but the presence or not of glycerol does not appear to matter.

Cryo Scanning Electron Microscopy (Cryo-SEM)

Figure 5 shows the microphotographs of the biscuits studied using Cryo-SEM. It can be observed how the biscuits are formed by a protein-sugar matrix where the starch granules are embedded within the matrix. During the baking process, the fat melts and coats the flour particles, making it difficult for them to hydrate and form bonds. Due to the fact that the formulation contains a great amount of sugar and insufficient water, many of the starch granules do not gelatinize, as is also the case in the study by Pareyt and Delcour (2008) on the influence that the different components of the flour exert on the quality of the biscuits. It should be pointed out that the biscuit made using the MCH emulsion (Figure 5F) has a matrix that is held together by a more continuous phase which coats the starch granules, as occurs in the control biscuit (Figure 5A); in the case of the biscuits made with the HPMC and MC emulsions (Figures 5B and D), the starch granules are looser and less embedded in the matrix. This leads to the control and MCH biscuits having a different textural profile to the HPMC and MC biscuits, which had higher values of hardness.

On the other hand, it may be appreciated that the presence of glycerol in the formulation did not produce any noticeable difference in the various samples, which coincides with what was found in the textural studies.

Acceptability

Figure 6 shows the results obtained from the assessment of sample acceptability. As regards the odour and sweetness attributes, the best evaluated samples were the biscuits elaborated with cellulose emulsions, although no differences ($p>0.05$) were found between the control biscuit and those made with the HPMC and MCH emulsions. For the crispness attribute no significant differences ($p>0.05$) were found among all the samples. The colour, taste and overall acceptability attributes followed similar trends; the samples elaborated with cellulose emulsions (MC, HPMC and MCH) obtained better scores ($p<0.05$) than the control biscuit. Only hardness was best evaluated for control biscuit than for the cellulose elaborated biscuits. In general, the biscuits with cellulose emulsions were the best accepted, as compared with the control biscuit, which scored the worst in five of seven attributes. In view of the results, the consumers preferred the biscuits made with emulsions containing cellulose ethers most than the control biscuits.

CONCLUSIONS

The emulsions prepared using cellulose ethers represent an excellent option for reducing the fat content in biscuits, as they exhibited very similar technological characteristics to the control dough and, in addition, the biscuits made with these emulsions were accepted by consumers.

Although the emulsion prepared using MCH did not show itself to be thermically stable in previous studies, the results show that, when incorporated into doughs, this MCH cellulose ether could be the most suitable option for designing emulsions to replace fat in biscuits. The sample prepared using this cellulose exhibits a hardness that is similar to the control biscuit and is more readily accepted by the consumers, as is the case for all of the formulations containing cellulose emulsions. In addition, it should be pointed out that by using this formulation, no glycerol would be needed and so the biscuit's characteristic crispness can be preserved through the classic batter formulation. If the other cellulose emulsions (MC and HPMC) are used, the addition of glycerol would be advisable as it reduces the hardness of the biscuits.

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Table 1. Breaking force obtained from penetration test of doughs and biscuits.

Glycerol	**Sample**	**Force (N) (Dough)**	**Force (N) (Biscuit)**
Without glycerol	Control	2.46a (0.09)	39.18a (15.45)
	HPMC	5.95b (0.11)	69.87bc (20.90)
	MC	5.00c (0.21)	87.07eb (26.86)
	MCH	4.34d (0.01)	40.37a (18.70)
With glycerol	HPMC	3.10e (0.06)	58.90cd (22.66)
	MC	4.38d (0.28)	89.95e (21.01)
	MCH	4.38d (0.22)	45.00ad (16.68)

Values in parentheses are standard deviations.

^{abc}Means with different letter in each column indicate significant differences among the samples ($p < 0.05$) according to the Tukey test.

FIGURE LEGENDS

Figure 1. Texture profile curves of biscuit doughs (A: without glycerol; B: with glycerol) and biscuits (C: without glycerol; d: with glycerol) made with shortening (control) and cellulose emulsions) (Blue: control; pink: MC; red: HPMC and green: MCH).

Figure 2. Confocal laser scanner microscopy (CLSM). Images of stained emulsions with Rhodamine B and Nile Red (proteins and carbohydrates in red, fat in green). Magnification (60x). (A: HPMC, B: MC and C: MCH).

Figure 3. Confocal laser scanner microscopy (CLSM) of stained doughs with Rhodamine B and Nile Red (proteins and carbohydrates in red, fat in green, starch in black). Magnification 60x. (Control: A; HPMC: B, without glycerol; C, with glycerol; MC: D, without glycerol; E, with glycerol; MCH: F, without glycerol; G, with glycerol).

Figure 4. Cryo-SEM micrographs of the inner part of the doughs. Magnification 500x. (Control: A; HPMC: B, without glycerol; C, with glycerol; MC: D, without glycerol; E, with glycerol; MCH: F, without glycerol; G, with glycerol).

Figure 5. Cryo-SEM micrographs of the inner part of the biscuits. Magnification 500x. (Control: A; HPMC: B, without glycerol; C, with glycerol; MC: D, without glycerol; E, with glycerol; MCH: F, without glycerol; G, with glycerol).

Figure 6. Consumer acceptability of the different biscuits made with shortening (control) and cellulose emulsions with glycerol (Blue: control; pink: MC; red: HPMC and green: MCH). (^{a,b} Different letters in each attribute indicate significant differences among the samples using Tukey test ($p<0.05$)).